APLICACIONES DE LA CITOMETRÍA DE FLUJO EN LA PRÁCTICA CLÍNICA

ASOCIACIÓN BIOQUÍMICA ARGENTINA – GRUPO RIOPLATENSE DE CITOMETRIA DE FLUJO

PATRONES INMUNOFENOTÍPICOS EN MÉDULA ÓSEA NORMAL / MIELOPOYESIS

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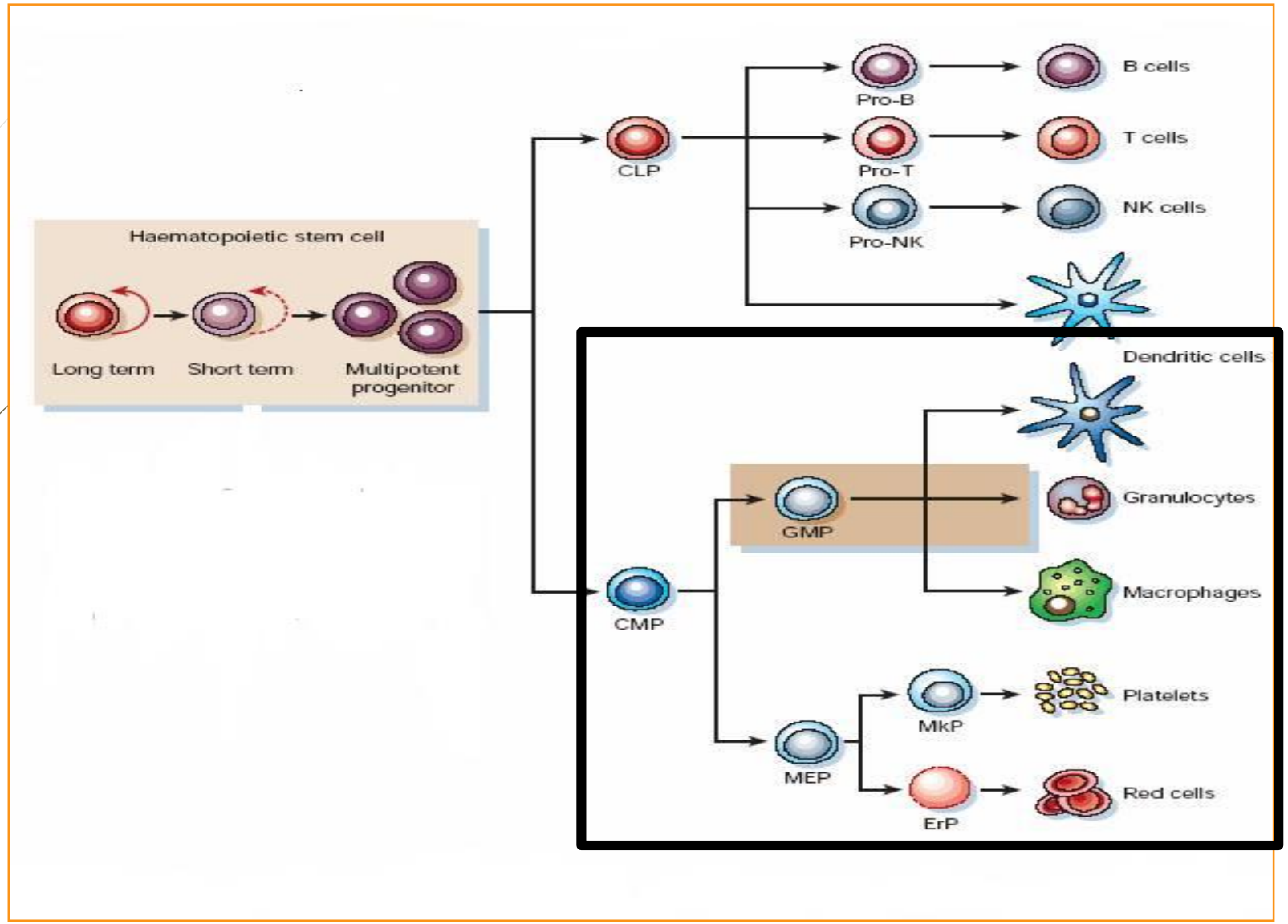

Haematopoietic stem cell
Long term
Short term
Multipotent progenitor
CLP
Pro-B
Pro-T
Pro-NK
B cells
T cells
NK cells
Dendritic cells
GMP
CMP
Granulocytes
Macrophages
MEP
MkP
ErP
Platelets
Red cells

Patrones de maduración mieloide Estrategia para valoración por CFM

- **Estudio de la población CD34+**
- **Estudio de la población CD34-, CD117+, HLA-DR+**
- **Estudio de las diferentes líneas celulares en su maduración**
  - **Neutrófilos**
  - **Monocitos**
  - **Eritroblastos**
  - **Basófilos**
  - **Eosinófilos**
  - **Células dendríticas**
  - **Megacariocitos**
  - **Mastocitos**

Maduración es un proceso continuo con progresión gradual entre los diferentes estadíos de maduración

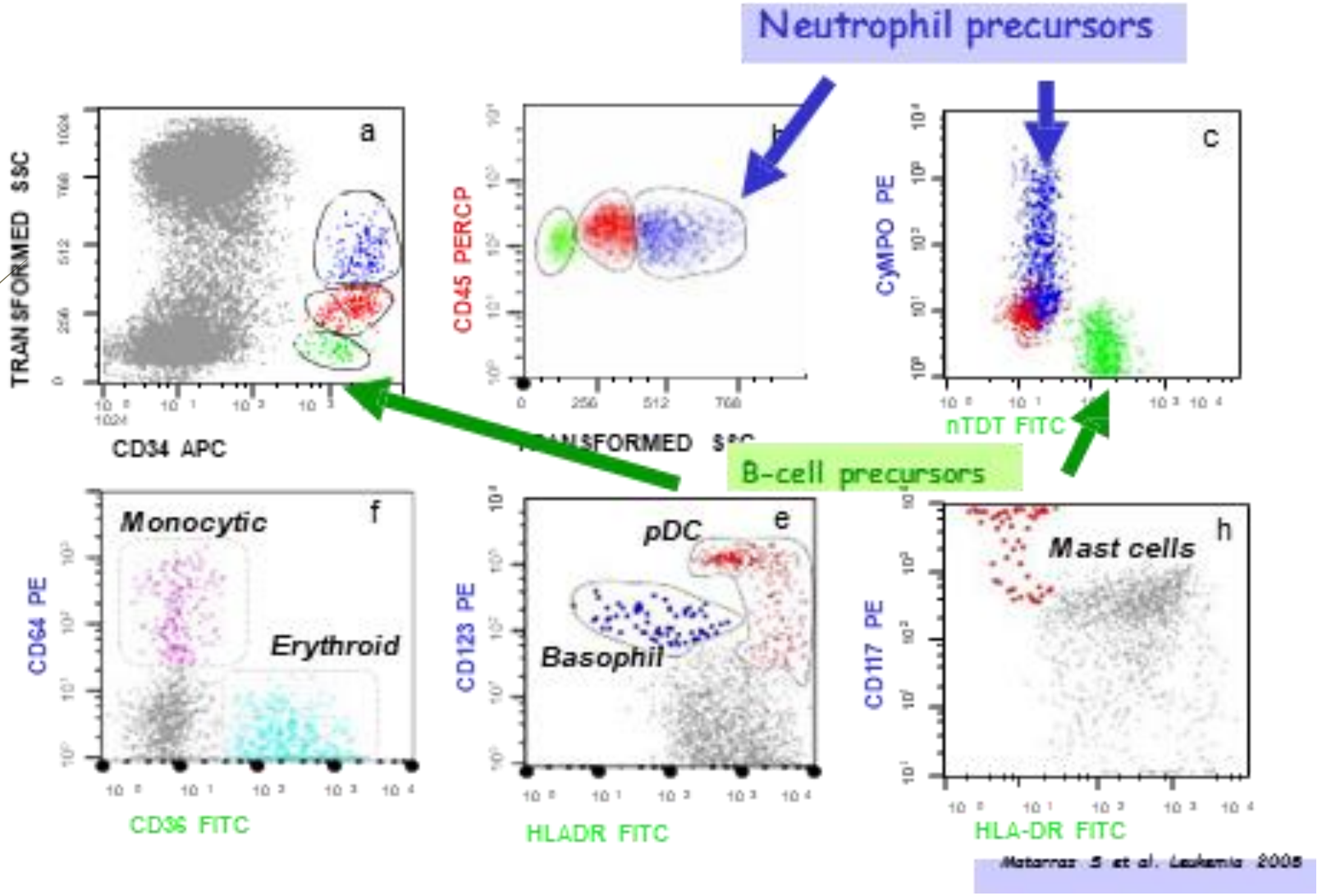
Frecuencia de progenitores mieloides CD34+ en MO

CELL LINEAGE	FREQUENCY
Erythroid	15% (5%-35%)
Megakaryocytic	<1%
Neutrophil	31% (12-39%)
Eosinophil	<1%
Basophil	<1% (<1%-3%)
Monocytic	5% (3%-15%)
Mast cell	<1% (<1%)
pDC	6% (1%-15%)
B-Lymphoid	23% (<1%-45%)
CD7+ (Early T-lymphoid?)	11% (5-18%)
Total:	92%

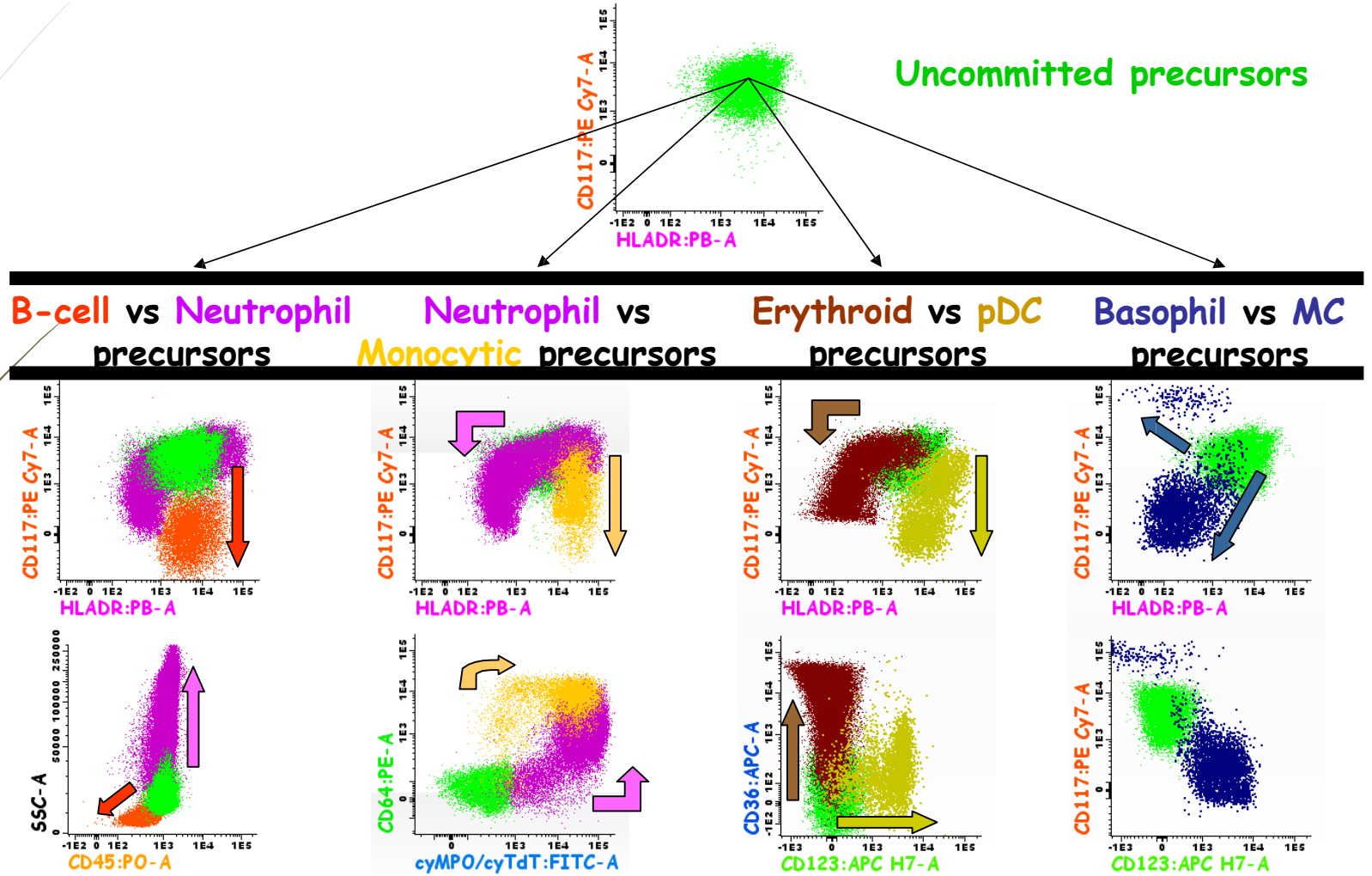
Inmunofenotipo de las progenitores mieloides CD34+

CELL LINEAGE	SSC	IMMUNOPHENOTYPE
Erythroid	stable	CD36+ CD105+, CD64-
Megakaryocytic	high	CD61+
Neutrophil	high	CyMPO+,
Eosinophil	high	CyEPO+
Basophil	low	$CD123^{hi}$ CD203c+, $CD117^{lo}$
Monocytic	stable	CD64+
Mast cell	low	$CD117^{hi}$
pDC	stable	CD36+ $CD123^{hi}$ $HLADR^{hi}$

Identificación de distintos compartimientos de células CD34+ por CF. Análisis bidimensional

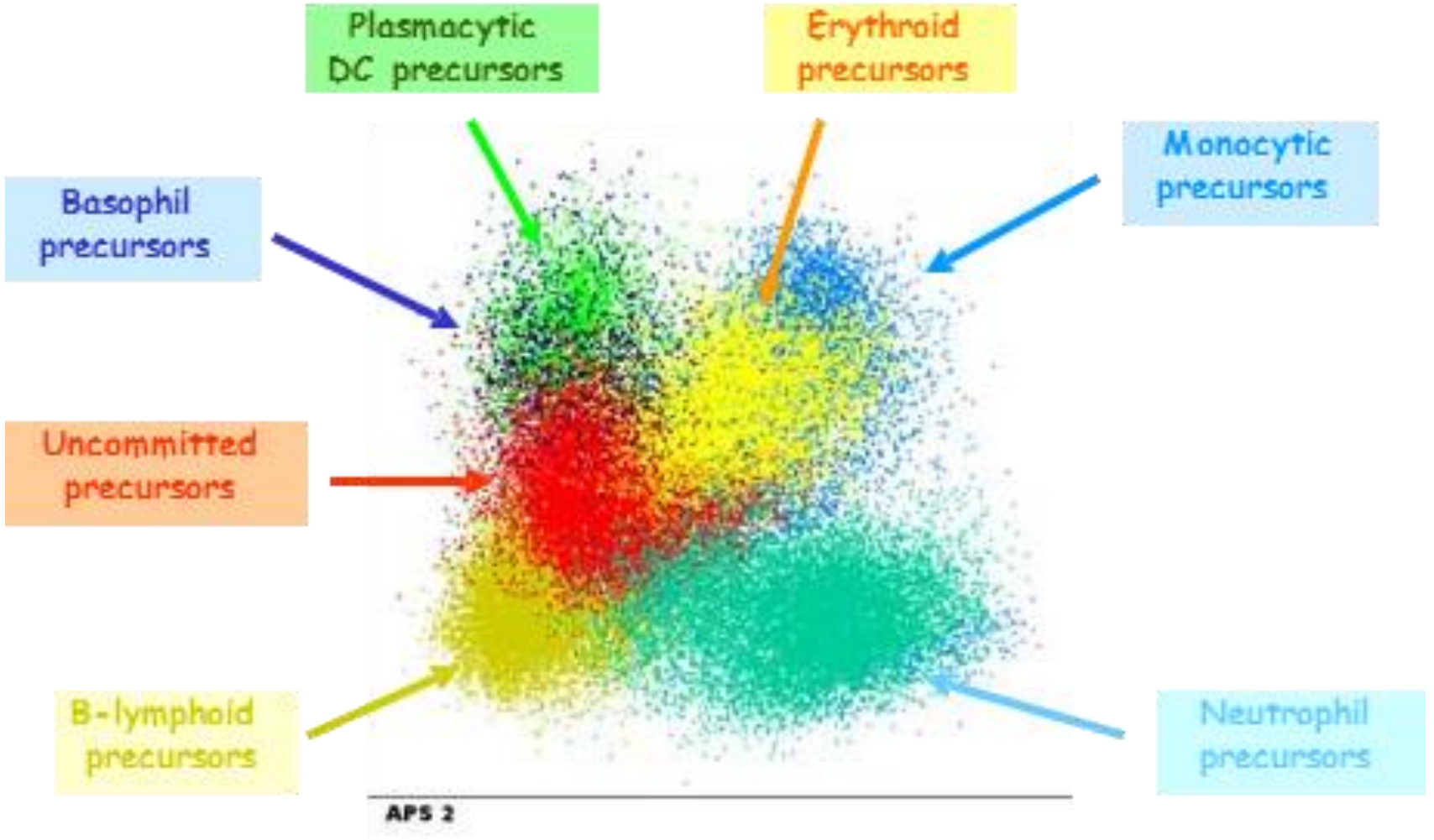


Progenitores CD34+ y/o CD117+



EuroFlow

Identificación de distintos compartimientos de células CD34+ por CFM
Análisis multivariante



EuroFlow

Maduración de líneas mieloides utilizando Euroflow

Multi-tube EuroFlow classification panel for AML/MDS

Responsible scientist: VHJ van der Velden

Tube	Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	Aim**
AML/ MDS									
1	**HLADR**	**CD45**	CD16	CD13	**CD34**	**CD117**	CD11b	CD10	Diagnosis and subclassification of AML and PNH especially focused on neutrophilic lineage
2	**HLADR**	**CD45**	CD35	CD64	**CD34**	**CD117**	IREM2	CD14	Diagnosis and subclassification of AML and PNH especially focussed on monocytic lineage
3	**HLADR**	**CD45**	CD36	CD105	**CD34**	**CD117**	CD33	CD71	Diagnosis and subclassification of AML especially focused on erythroid lineage
4	**HLADR**	**CD45**	nuTdT	CD56	**CD34**	**CD117**	CD7	CD19	Aberrant expression of lymphoid-associated markers and abnormal lymphoid maturation

* Further information about the markers and the availability of hybridoma clones is summarized in Appendix A. Backbone markers are indicated in bold; nu= nuclear.
** The described marker combinations might also be applied for disease staging and monitoring of treatment effectiveness (MRD diagnostics)

Maduración de línea neutrófilo

Multi-tube EuroFlow classification panel for AML/MDS

Responsible scientist: VHJ van der Velden

Tube	Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	Aim**
AML/ MDS									
1	**HLADR**	**CD45**	CD16	CD13	**CD34**	**CD117**	CD11b	CD10	Diagnosis and subclassification of AML and PNH especially focused on neutrophilic lineage

* Further information about the markers and the availability of hybridoma clones is summarized in Appendix A. Backbone markers are indicated in bold; nu= nuclear.
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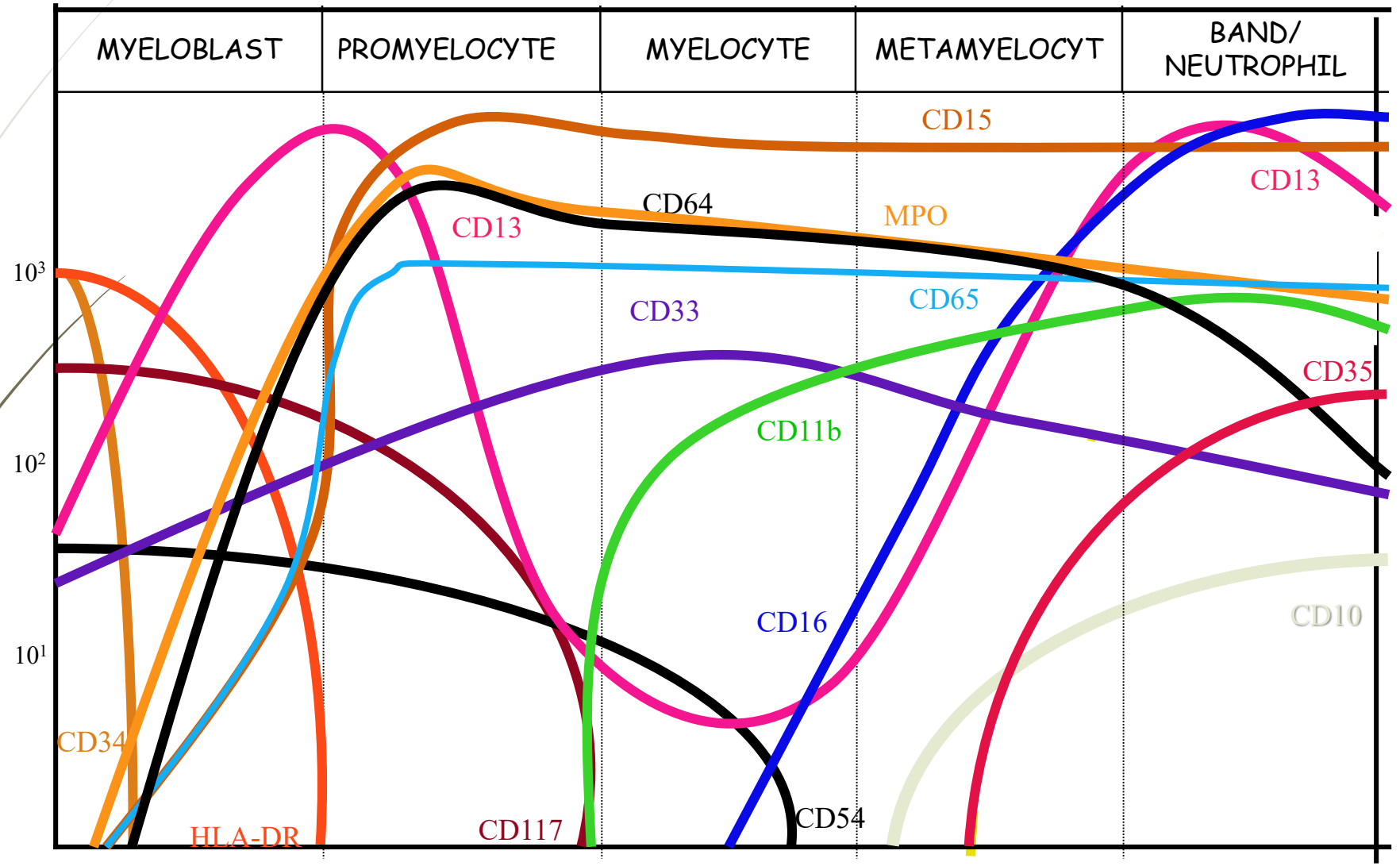
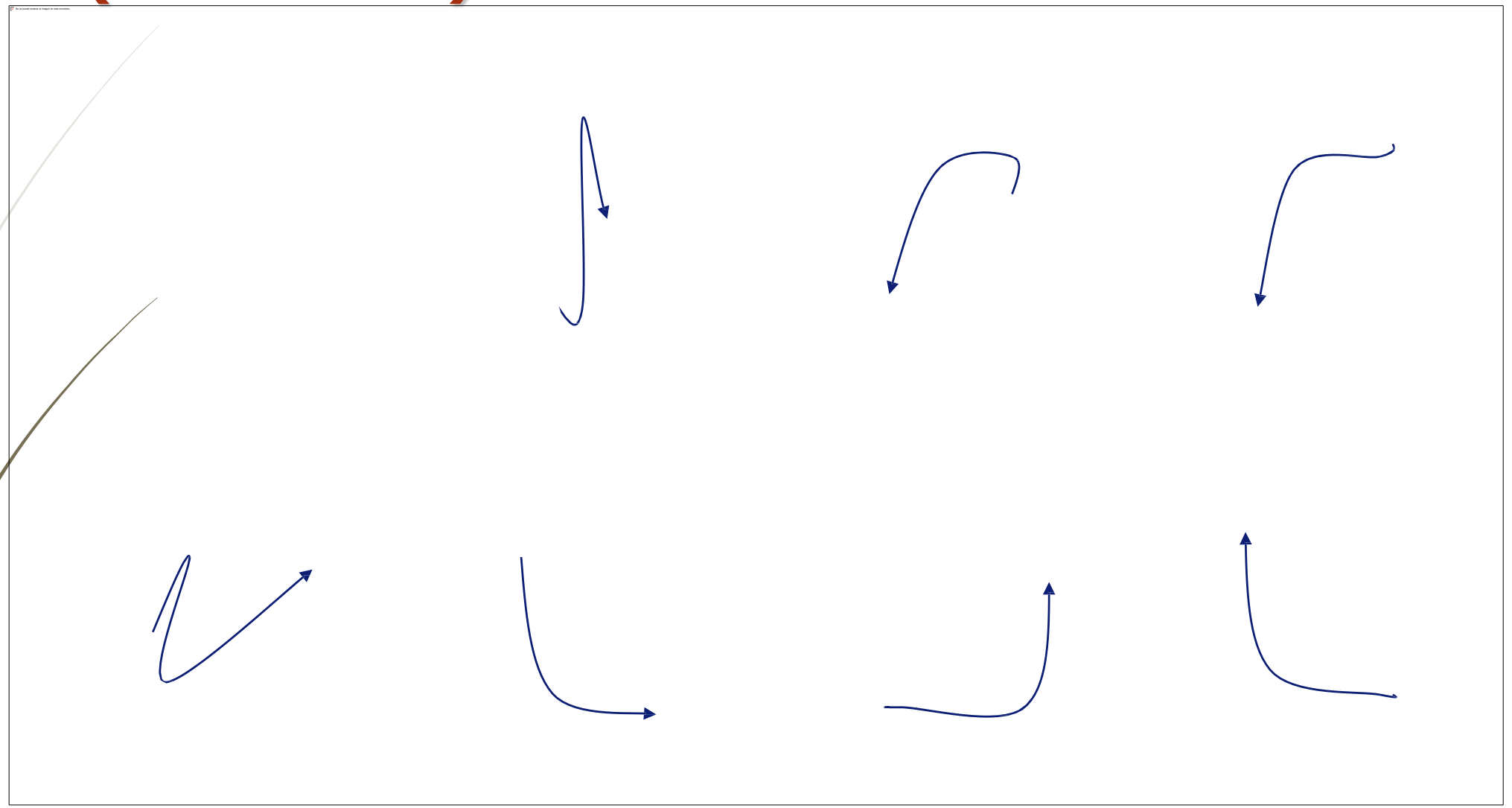
Cambios fenotípicos durante la diferenciación a neutrófilo

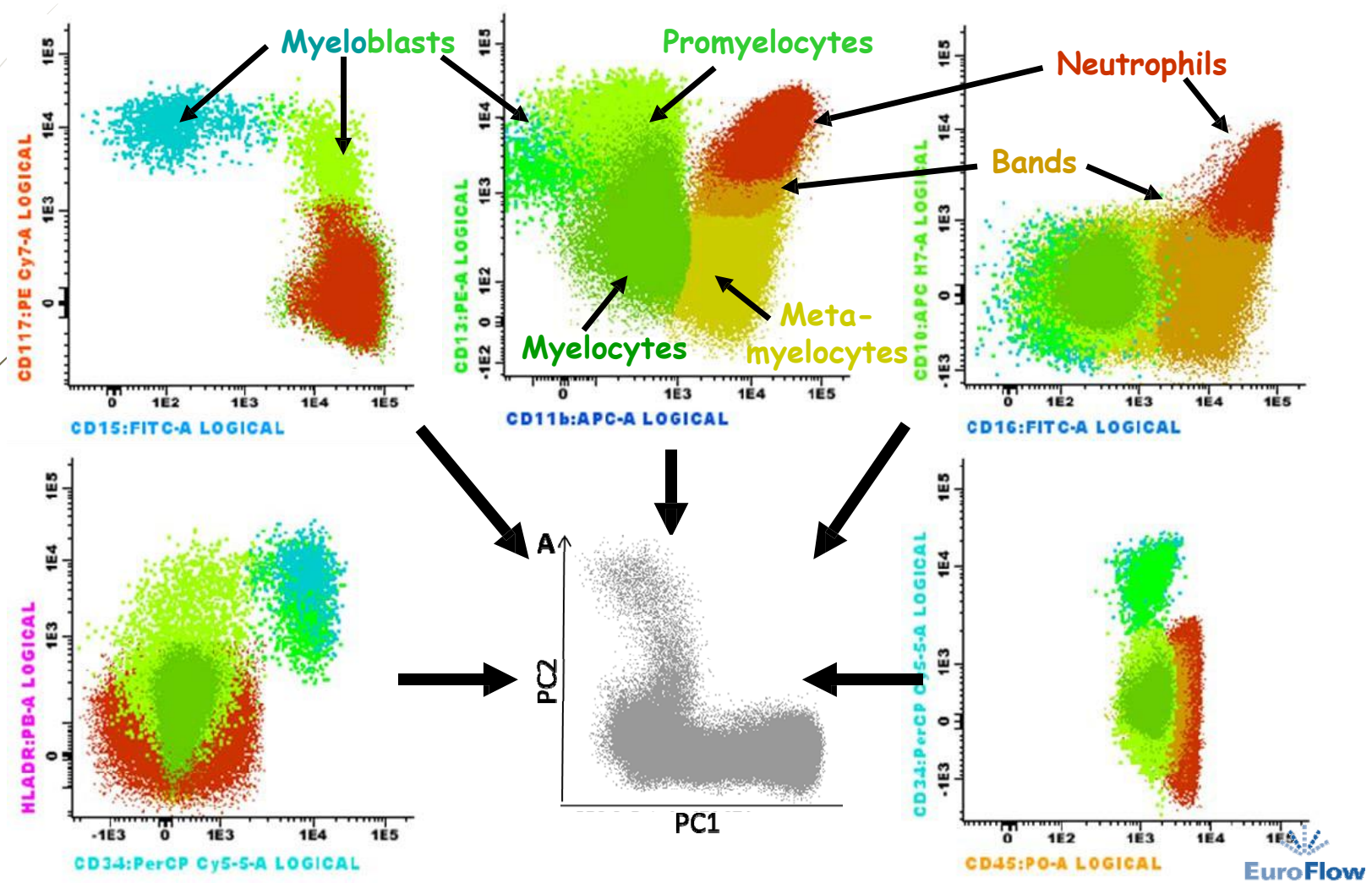


Diferenciación a neutrófilo (tubo AML1)



van der Velde. ESCCA 2012

Maduración normal a neutrófilo



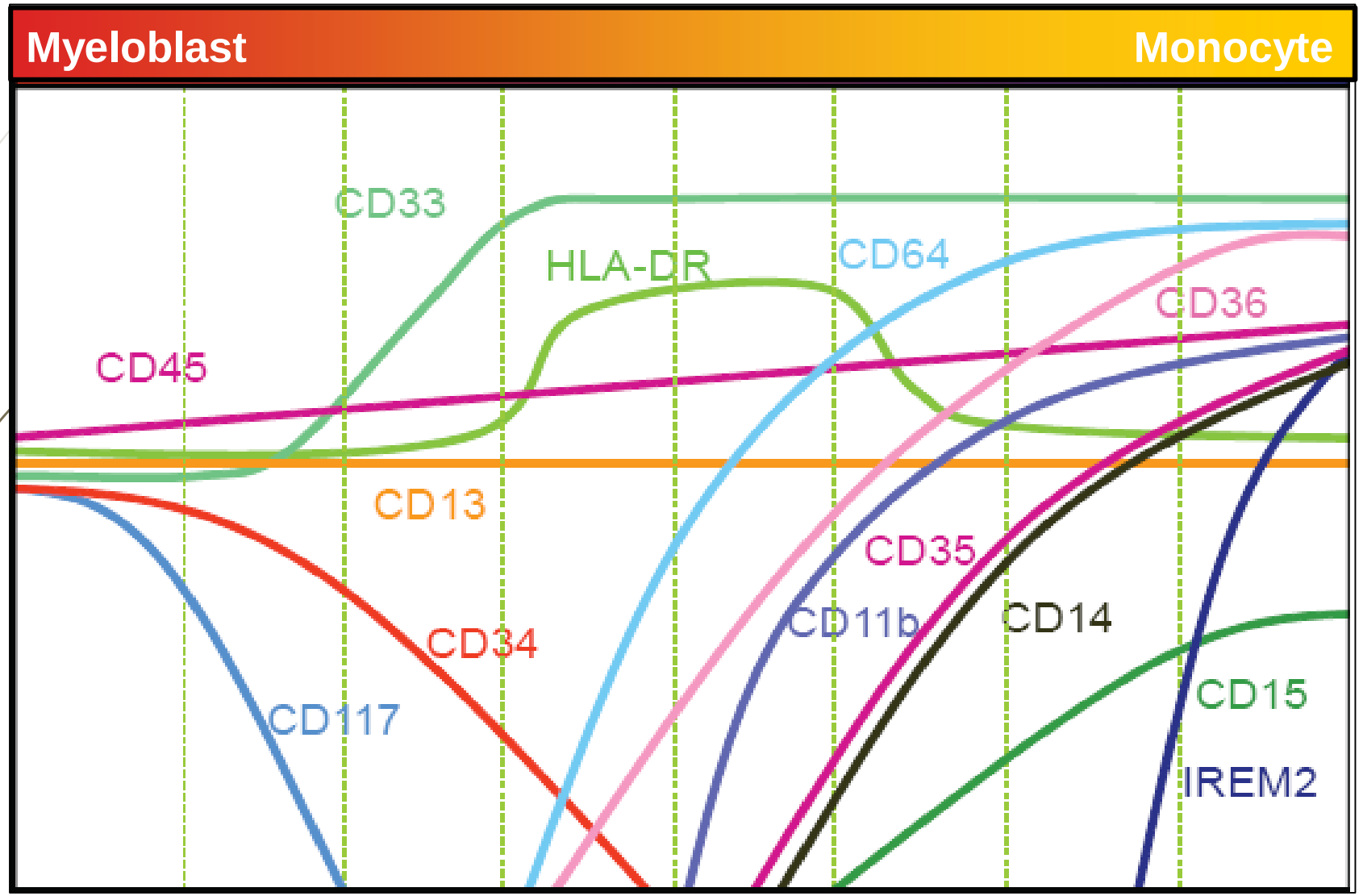
Multi-tube EuroFlow classification panel for AML/MDS

Responsible scientist: VHJ van der Velden

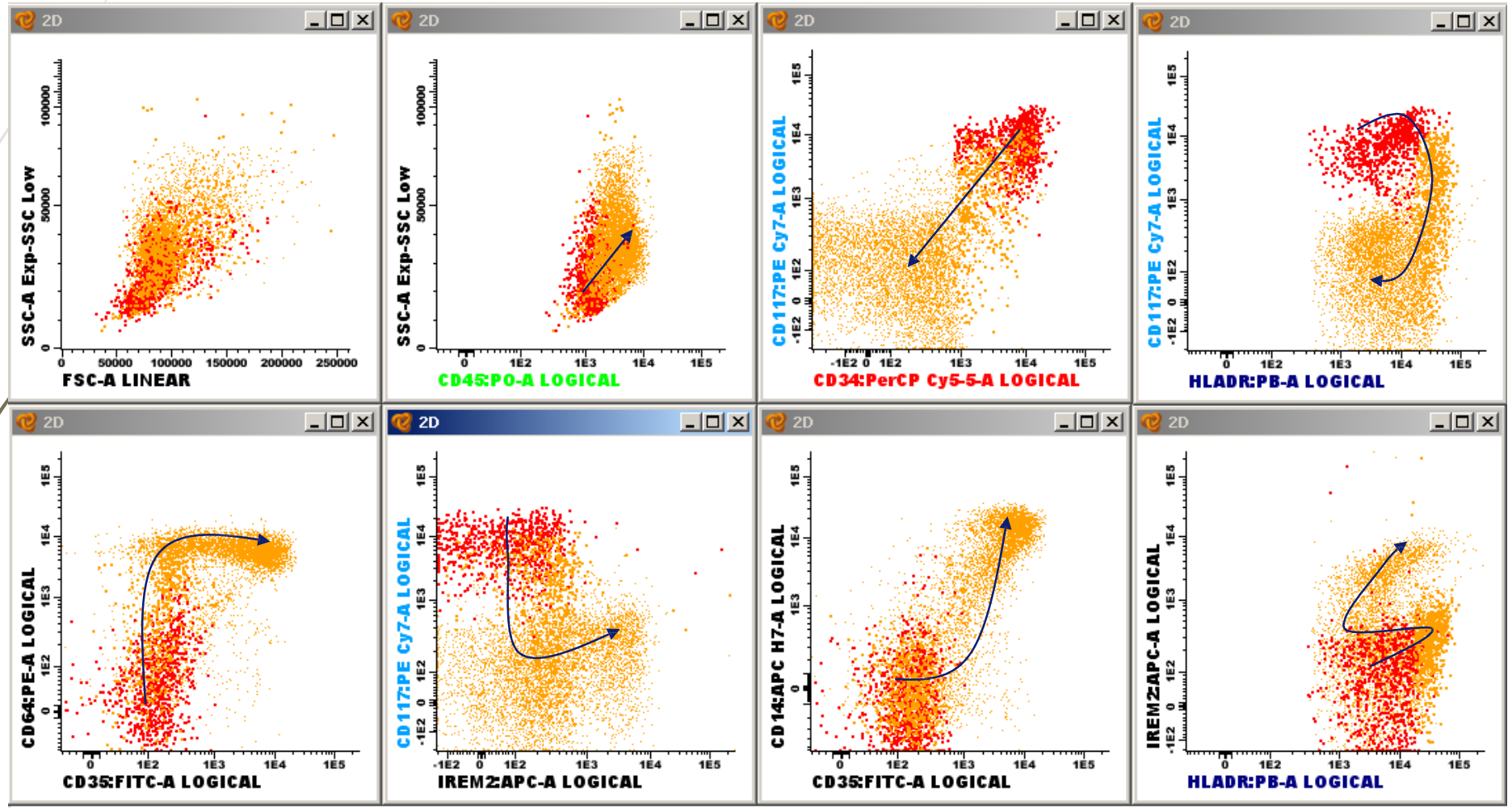
Tube	Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	Aim**
AML/ MDS									
2	**HLADR**	**CD45**	CD35	CD64	**CD34**	**CD117**	IREM2	CD14	Diagnosis and subclassification of AML and PNH especially focussed on monocytic lineage

* Further information about the markers and the availability of hybridoma clones is summarized in Appendix A. Backbone markers are indicated in bold; nu= nuclear.
** The described marker combinations might also be applied for disease staging and monitoring of treatment effectiveness (MRD diagnostics)

Diferenciación monocítica



Diferenciación monocítica (tubo AML2)



van der Velde. ESCCA 2012

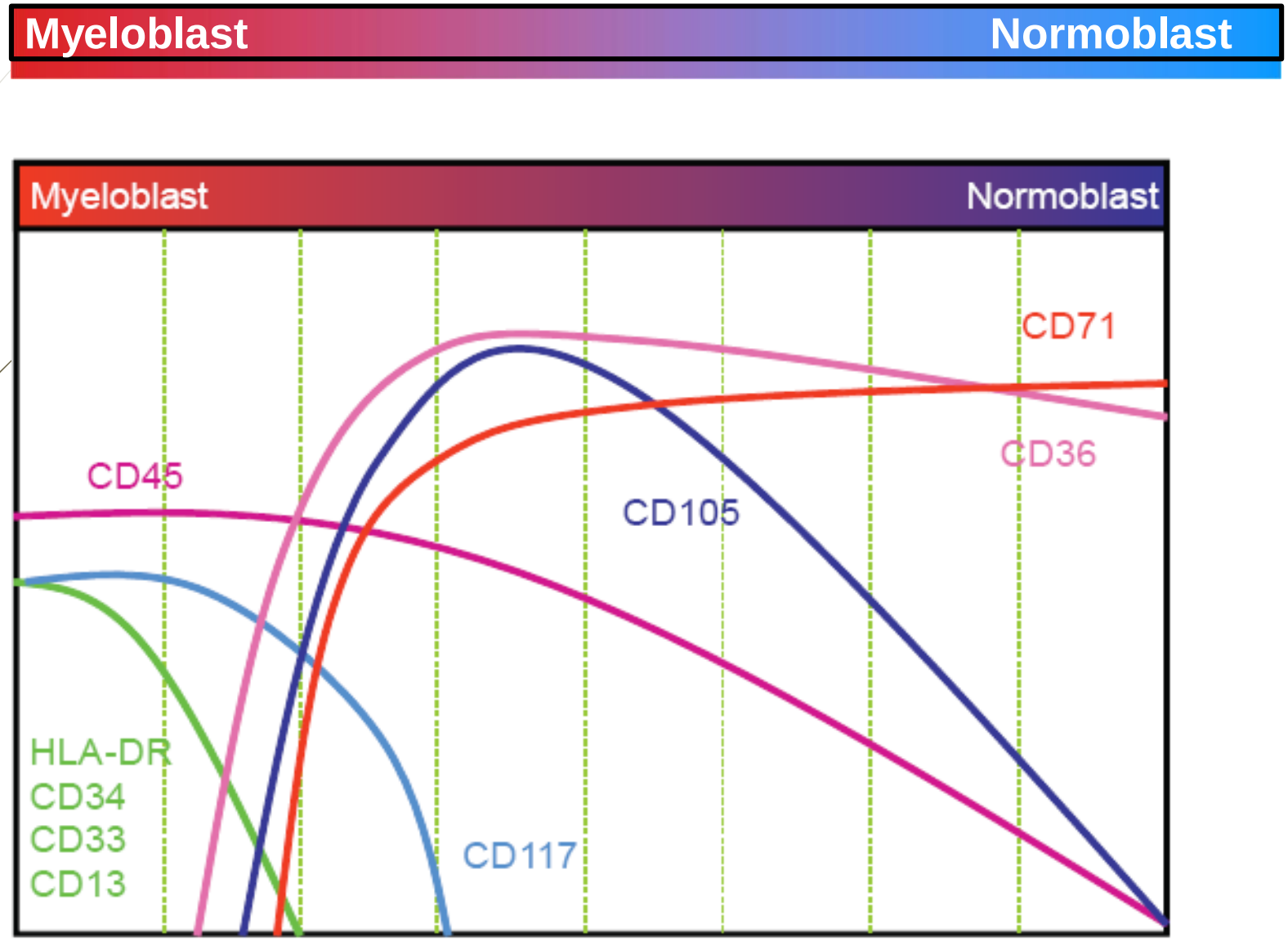
Multi-tube EuroFlow classification panel for AML/MDS

Responsible scientist: VHJ van der Velden

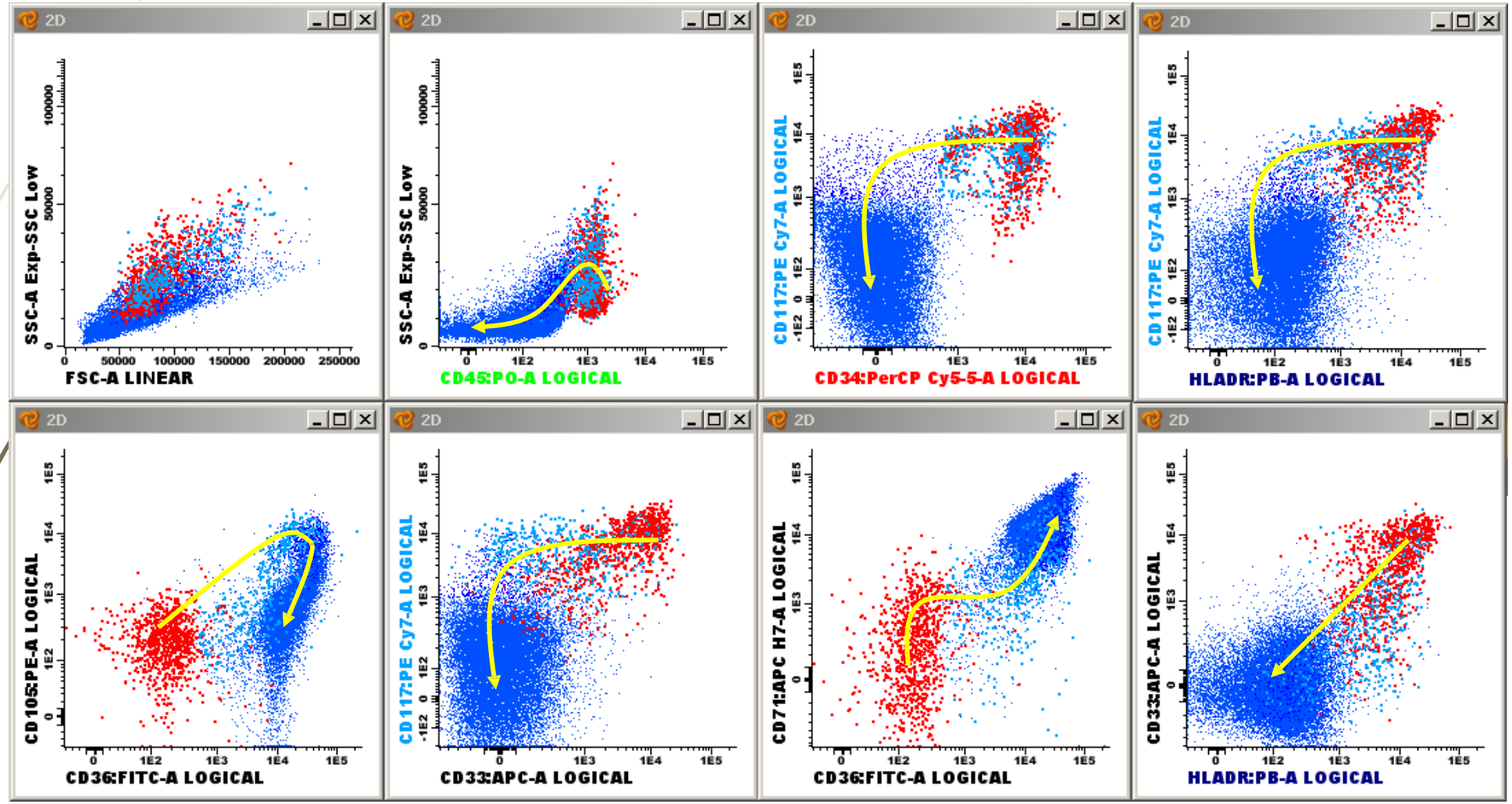
Tube	Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	Aim**
AML/ MDS									
3	**HLADR**	**CD45**	CD36	CD105	**CD34**	**CD117**	CD33	CD71	Diagnosis and subclassification of AML especially focused on erythroid lineage

* Further information about the markers and the availability of hybridoma clones is summarized in Appendix A. Backbone markers are indicated in bold; nu= nuclear.
** The described marker combinations might also be applied for disease staging and monitoring of treatment effectiveness (MRD diagnostics)

Diferenciación eritroide



Diferenciación eritroide tubo AML3

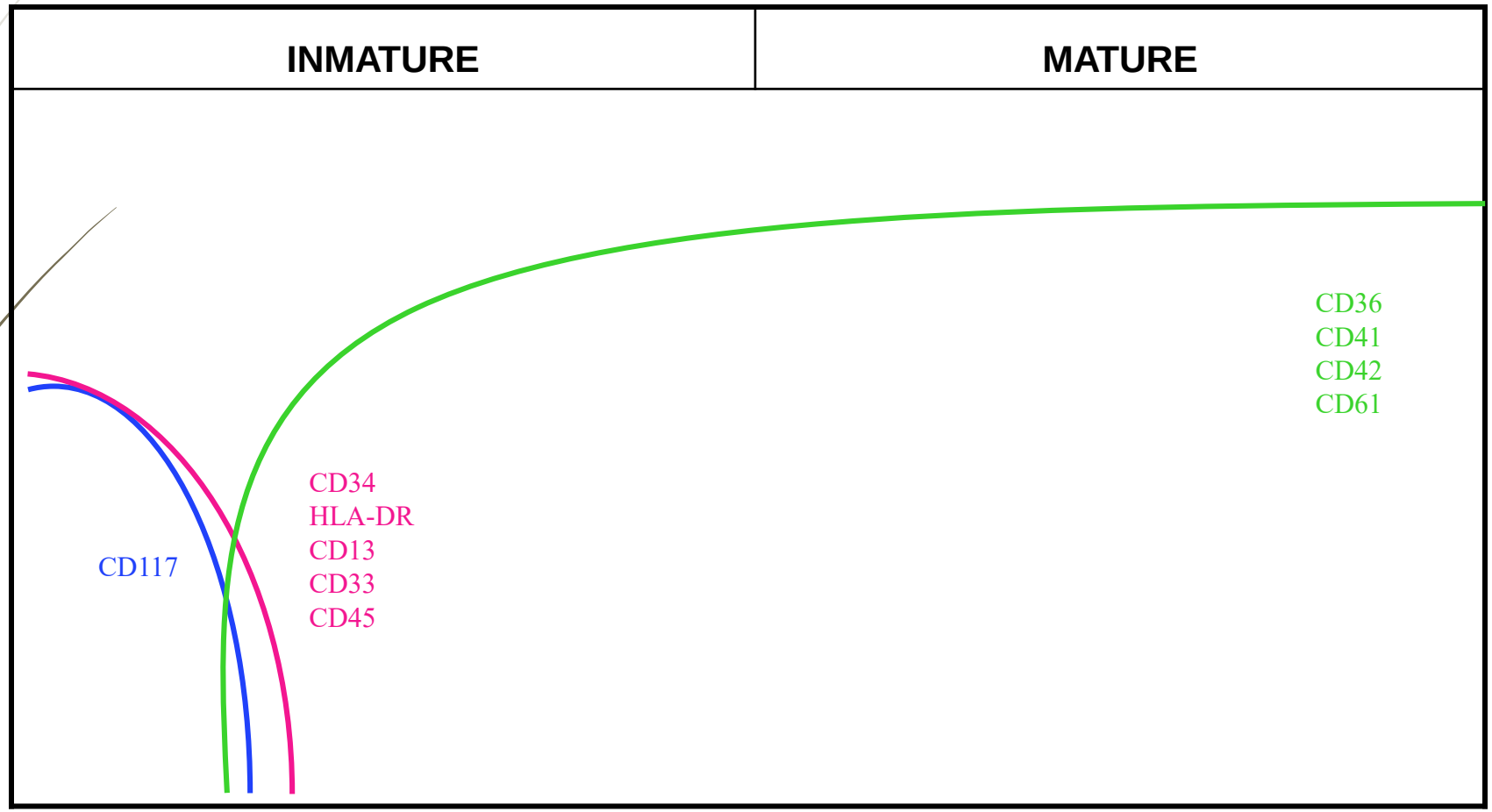


van der Velde. ESCCA 2012

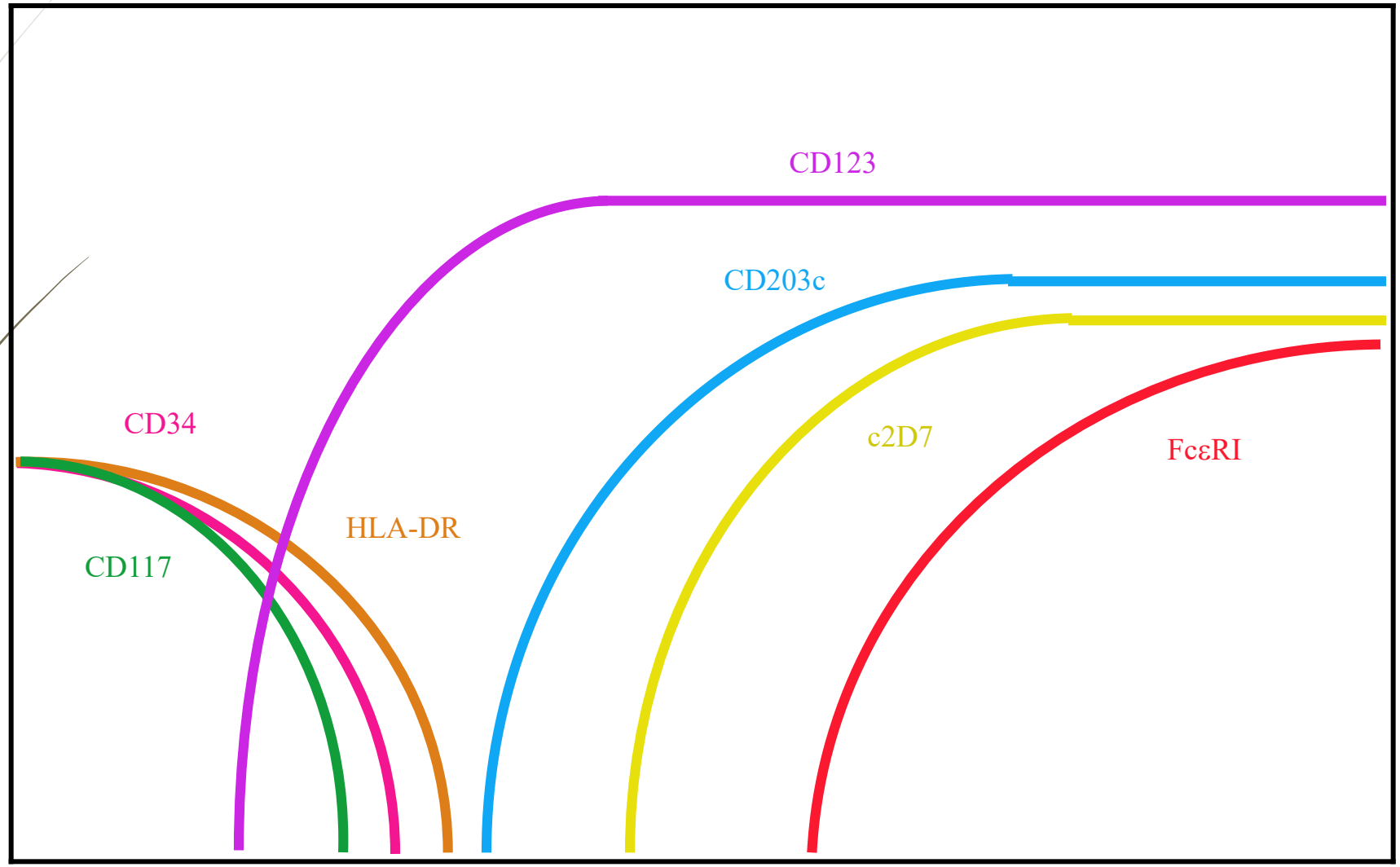
Caracterización fenotípica de otras líneas mieloides

Tube	PacB	PacO	FITC	PE	PerCPCy5.5	PECy7	APC	APCH7	Aim
1	HLADR	CD45	CD16	CD13	CD34	CD117	CD11b	CD10	Diagnosis and classification, neutrophilic maturation, PNH
2	HLADR	CD45	CD35	CD64	CD34	CD117	CD300e (IREM2)	CD14	Diagnosis and classification, monocytic maturation, PNH
3	HLADR	CD45	CD36	CD105	CD34	CD117	CD33	CD71	Diagnosis and classification, erythroid maturation
4	HLADR	CD45	NuTdT	CD56	CD34	CD117	CD7	CD19	Aberrant expression of lymphoid markers, abnormal B lymphoid maturation
5	HLADR	CD45	CD15	NG2	CD34	CD117	CD22	CD38	Aberrant marker expression, stem cells
6	HLADR	CD45	CD42a and CD61	CD203c	CD34	CD117	CD123	CD4	Diagnosis and classification of AML Megakaryocytic, basophilic, and plasmacytoid dendritic cell lineages
7	HLADR	CD45	CD41	CD25	CD34	CD117	CD42b	CD9	Characterization of megakaryoblastic leukemia, and systemic mastocytosis

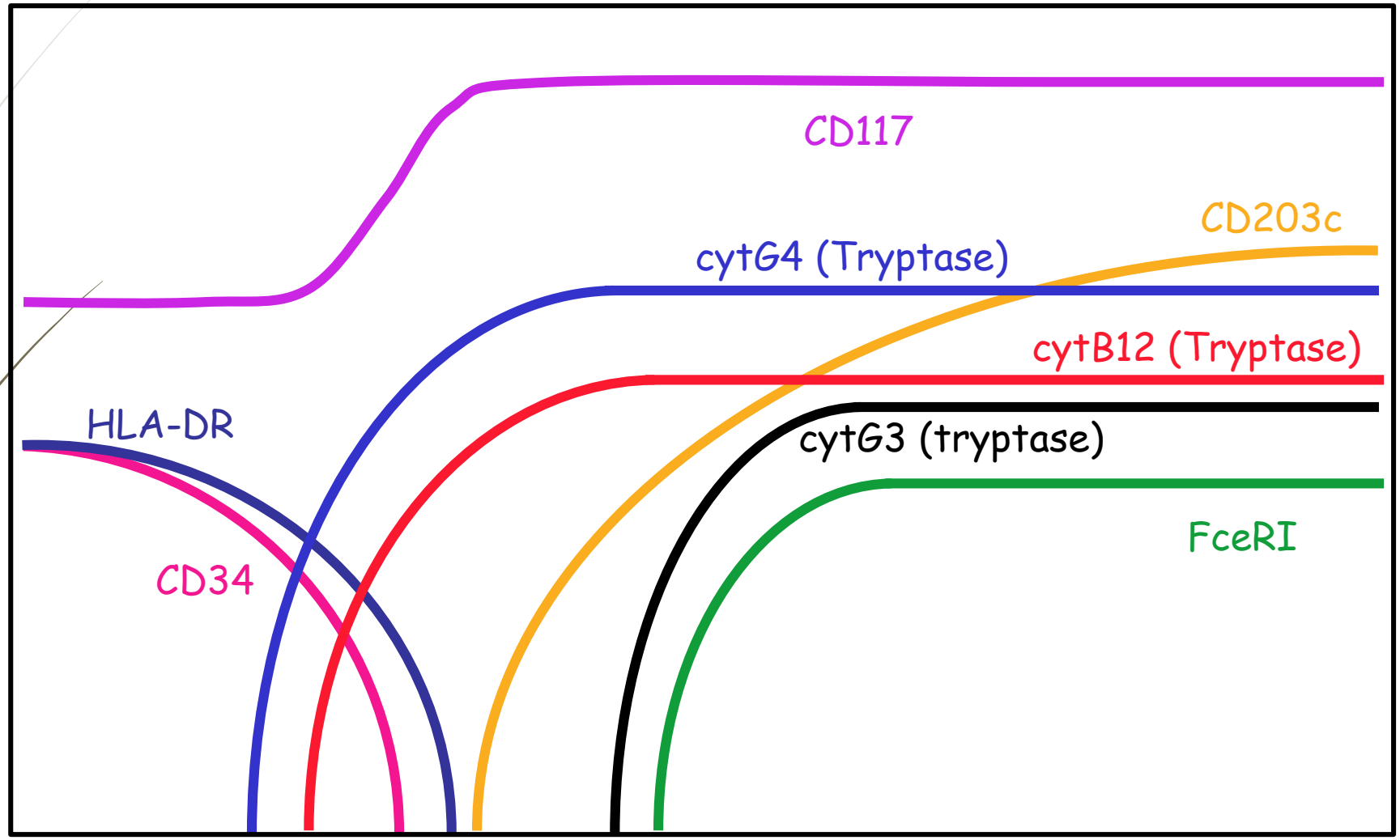
Cambios fenotípicos durante la diferenciación megacariocítica



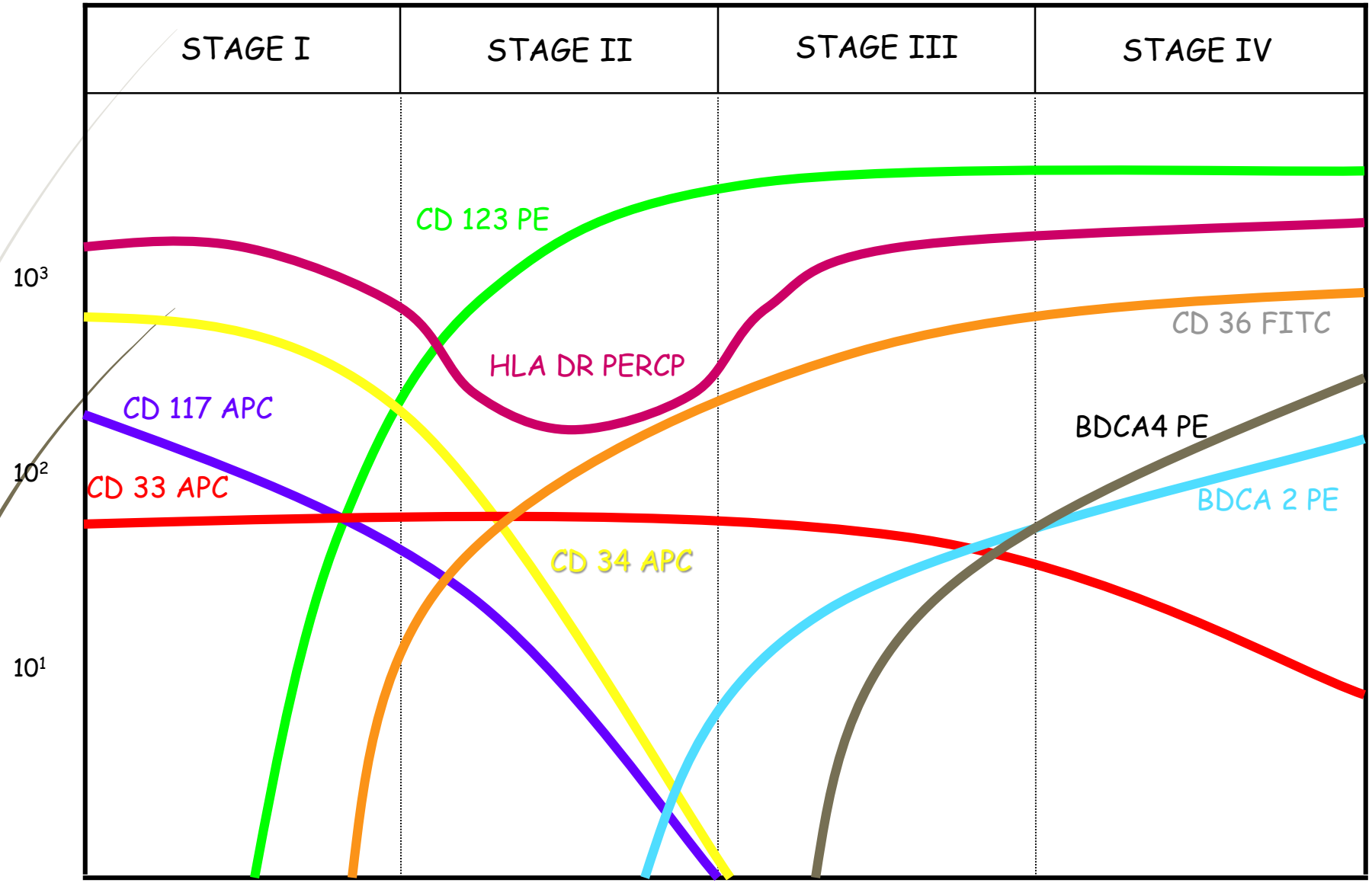
Cambio fenotípico durante la diferenciación a basófilo



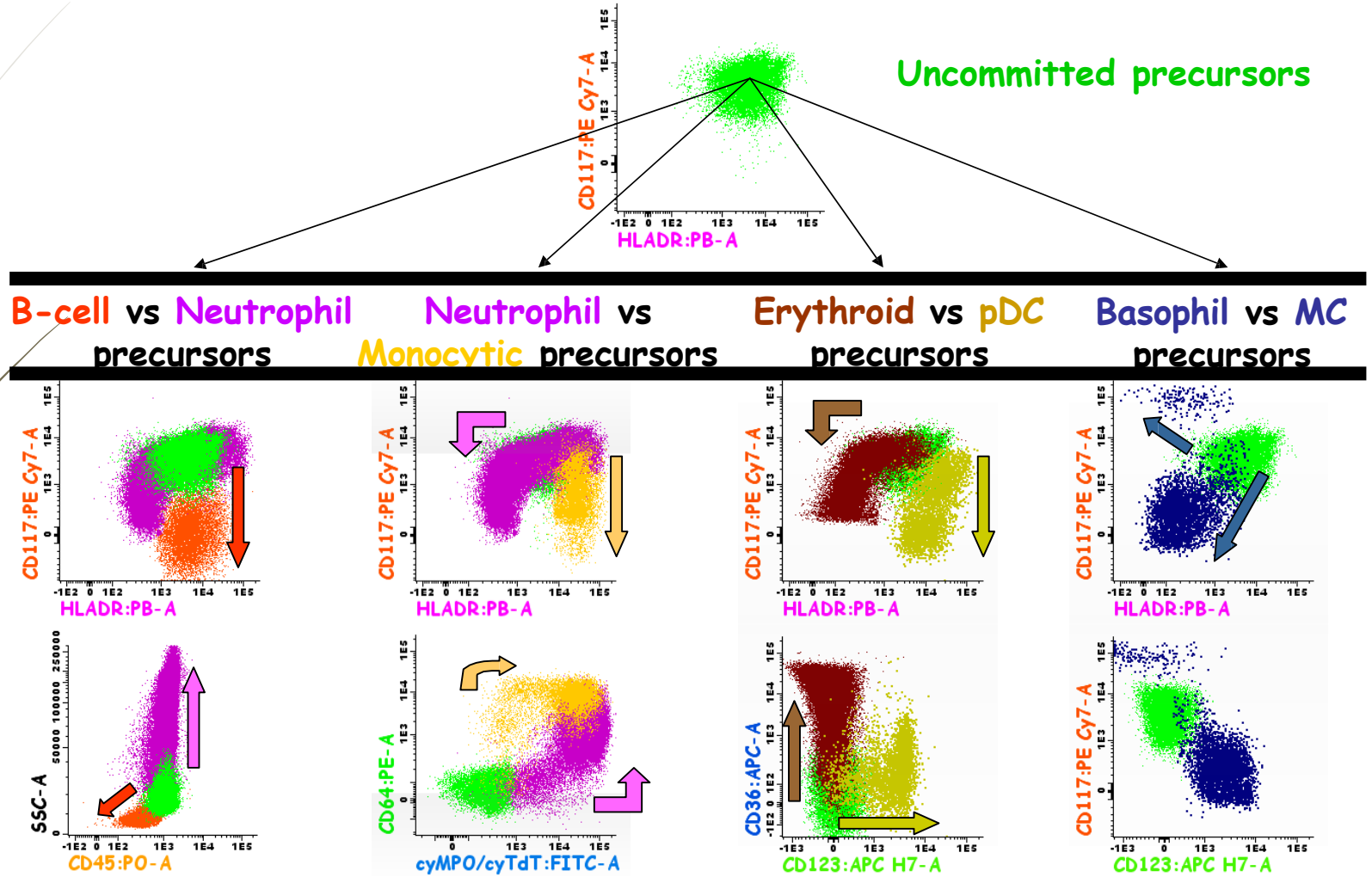
Cambios fenotípicos durante la diferenciación a mastocito



Cambios fenotípicos durante la maduración de DC plasmocitoides



Progenitores CD34+ y/o CD117+

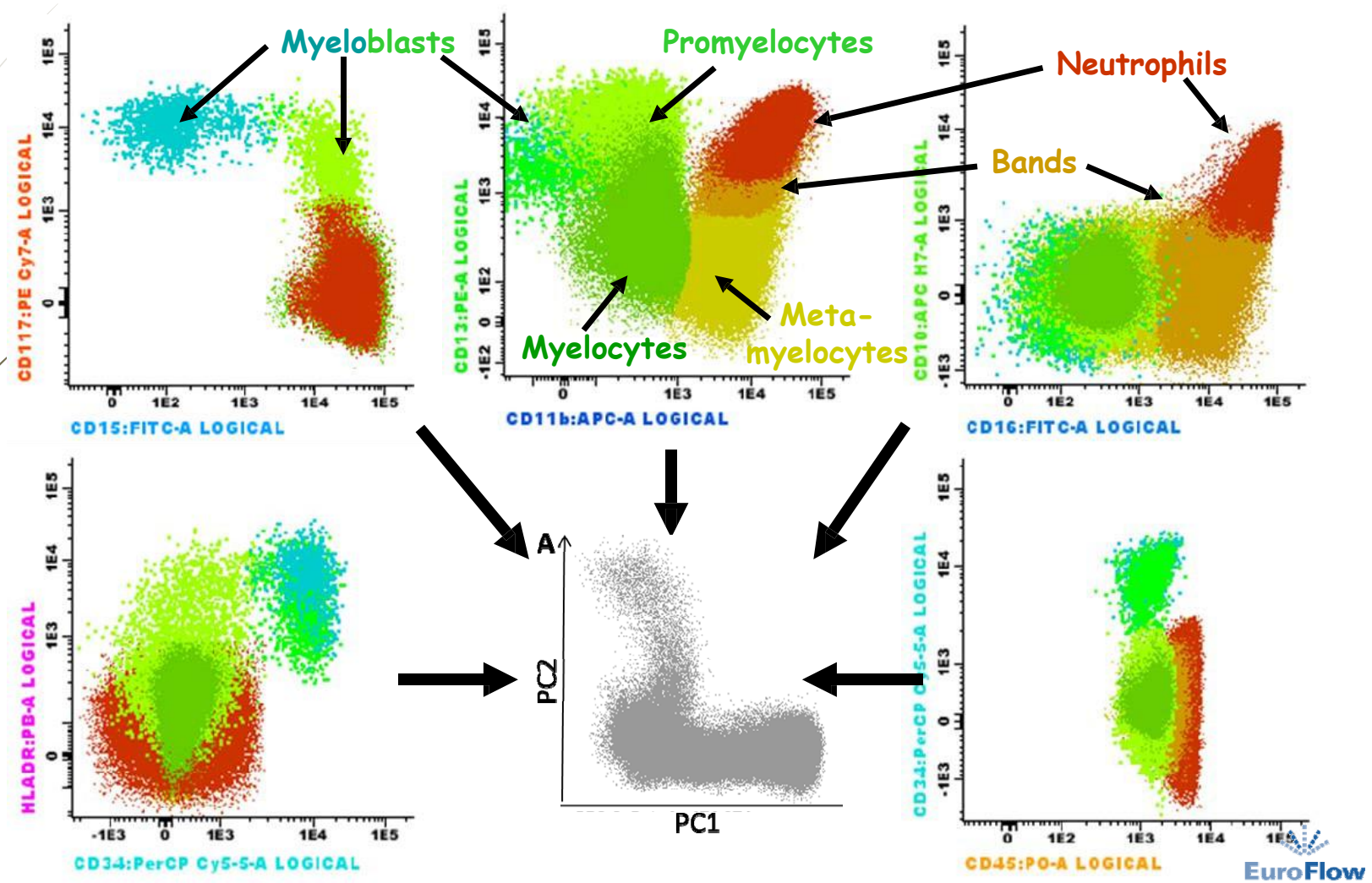


EuroFlow

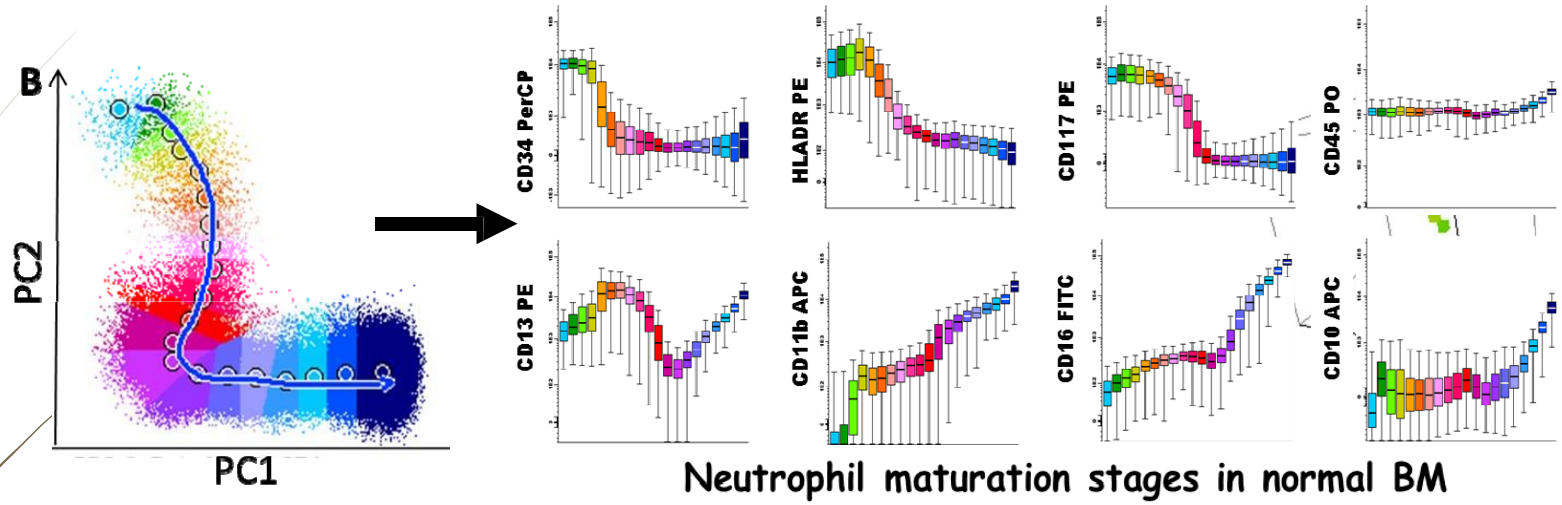
Maduración normal vs neoplásica

- Clasificación de la población leucémica de una leucemia aguda según su linaje y estadío de maduración
- Identificación de fenotipos de maduración alteradas para el diagnóstico de SMD
- Valoración de enfermedad mínima residual en LAM

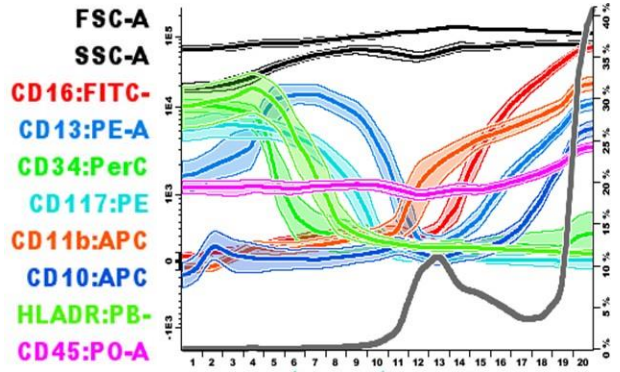
Maduración normal a neutrófilo



Maduración normal a neutrófilo



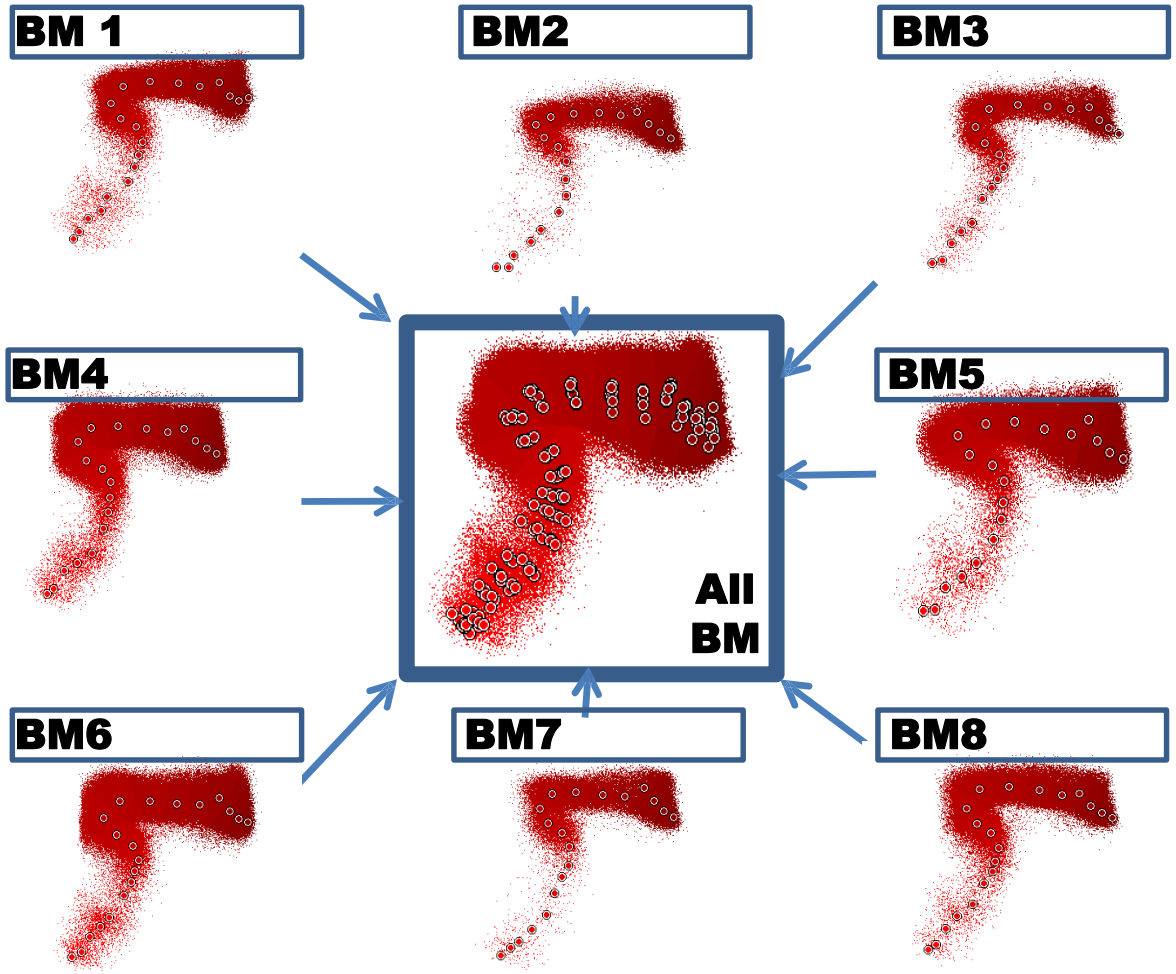
Neutrophil maturation stages in normal BM



Neutrophil maturation stages in normal BM

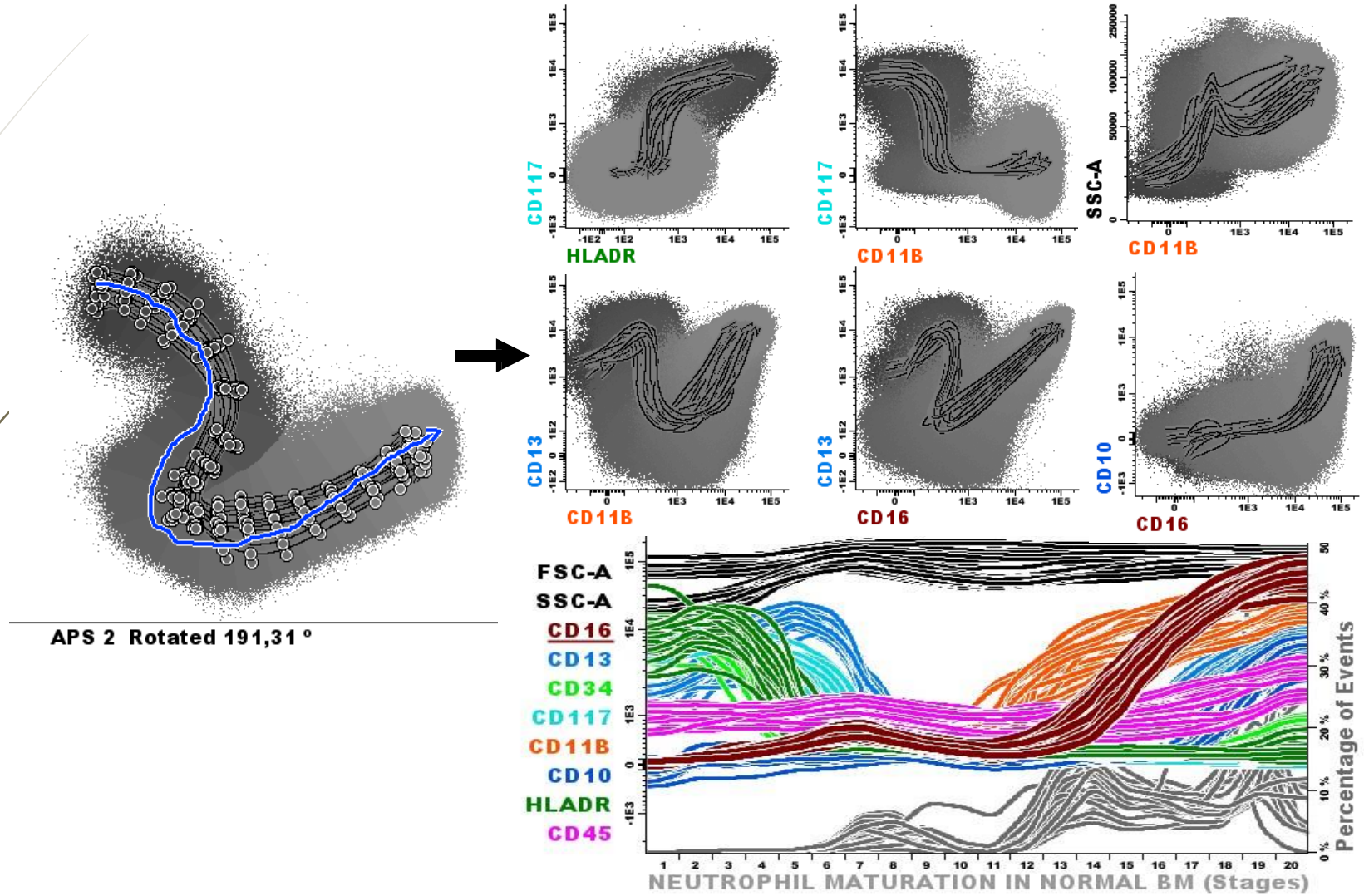
Sequential markers: CD34- DR- CD117- CD13- CD11b+ CD16+ CD13+ CD10+

Maduración a Neutrófilo en MO Normal basada en análisis de componentes principales de 10 parámetros



EuroFlow

Bases de datos de referencia de maduración a neutrófilo en MO Normal



N=23

EuroFlow

Conclusiones

- La CFM puede caracterizar los perfiles fenotípicos de las células normales en MO durante su maduración a diferentes líneas mieloides siendo muy útil para diagnosticar las LAM y caracterizar su línea y estado madurativo

- Existe una importante heterogeneidad en cuanto al análisis de la maduración mieloide patológica así como en la valoración de enfermedad residual por los distintos operadores

- Nuevas herramientas informáticas y probablemente la valoración simultánea de un mayor número de marcadores sean necesarias para mejorar la sensibilidad y especificidad de la técnica para este fin